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ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.*

(54) Title: TRANSGENIC PLANTS USED AS A BIOREACTOR SYSTEM

(57) **Abstract:** The present invention relates generally to the use of plants as bioreactors for the production of molecules having useful properties such as *inter alia* polymers, metabolites, proteins, pharmaceuticals and nutraceuticals. More particularly, the present invention contemplates the use of grasses, and even more particularly C4 grasses, such as sugar-cane, for the production of a range of compounds such as, for example, polyhydroxyalkanoates, pHBA, vanillin, indigo, adipic acid, 2-phenylethanol, 1,3-propandiol, sorbitol, fructan polymers and lactic acid as well as other products including, *inter alia*, other plastics, silks, carbohydrates, therapeutic and nutraceutic proteins and antibodies. The present invention further extends to transgenic plants and, in particular, transgenic C4 grass plants, capable of producing the compounds noted above and other products, and to methods for generating such plants. The ability to utilize the high growth rate and efficient carbon fixation of C4 grasses is advantageous, in that it obviates the significant growth penalties observed in other plants, and results in high yields of desired product without necessarily causing concomitant deleterious effects on individual plants. In addition, the C4 grass, sugarcane, is particularly advantageous, as in addition to the features common to all C4 grasses, this plant accumulates sucrose. This sucrose store provides a ready supply of carbon based compounds and energy which may further obviate any deleterious effects on the growth of the plant associated with the production of the product. The present invention provides, therefore, a bioreactor system comprising a genetically modified plant designed to produce particular metabolic or biosynthetic products of interest.

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/AU03/00903

| <b>A. CLASSIFICATION OF SUBJECT MATTER</b>  |   |  |
|---|---|--|
| Int. Cl. <sup>7</sup> : A01H 5/00   |   |  |
| According to International Patent Classification (IPC) or to both national classification and IPC   |   |  |
| <b>B. FIELDS SEARCHED</b>   |   |  |
| Minimum documentation searched (classification system followed by classification symbols)   |   |  |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched   |   |  |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)<br>WPIDS, MEDLINE, CAPLUS, AGRICOLA: polyhydroxyalkanoate, gramineae, poaceae, grass, grasses, sugarcane, saccharum, maize, zeo, sorghum, monocotyleda   |   |  |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>   |   |  |
| Category*   | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.  |
| X   | US 6 091 002 A (Asrar <i>et al</i> ) 18 July 2000<br>whole of document              | 1-7, 35-44, 54   |
| X   | WO 00/52183 A1 (Monsanto Company) 8 September 2000<br>whole of document             | 1-7, 35-44, 54   |
| X   | WO 01/23596 A2 (Pioneer Hi-Bred Internation, Inc) 5 April 2001<br>whole of document | 1-7, 35-44, 54   |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex   |   |  |
| <p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p> |   |  |
| Date of the actual completion of the international search<br>22 October 2003  |   | Date of mailing of the international search report<br>31 OCT 2003      |
| Name and mailing address of the ISA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>E-mail address: pct@ipaaustralia.gov.au<br>Facsimile No. (02) 6285 3929  |   | Authorized officer<br><br>GARETH COOK<br>Telephone No : (02) 6283 2541 |

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU03/00903

**C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.       |
|-----------|--|-----------------------------|
| X         | Snell KD <i>et al</i> , "Polyhydroxyalkanoate Polymers and Their Production in Transgenic Plants", <i>Metabolic Engineering</i> , January 2002, 4(1):29-40<br>whole of document.   | 1, 2, 5-7, 35-37, 39, 41-44 |
| X         | Hahn JJ <i>et al</i> , "Peroxisomes as Sites for Synthesis of Polyhydroxyalkanoates in Transgenic Plants", <i>Biotechnology Progress</i> , 1999, 15:1053-1057<br>whole of document   | 1 2, 5-7, 35-37, 42-44      |
| Y         | Brumbley SM <i>et al</i> , "Application of Biotechnology for Future Sugar Industry Diversification", <i>Proceedings of the Conference of the Australian Society of Sugar Cane Technologists</i> , 29 April to 1 May 2002, 24:40-46<br>pages 41 to 43 | 1-7, 35-44, 54              |
| Y         | Bohmert K <i>et al</i> , "Transgenic <i>Arabidopsis</i> plants can accumulate polyhydroxybutyrate to up to 4% of their fresh weight", <i>Planta</i> , 2000, 211:941-945<br>whole of document   | 1-7, 35-44, 54              |

## INTERNATIONAL SEARCH REPORT

International application No.

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**Box I** Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos :  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos :  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

**Box II** Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
Claims 1 to 4, 35 to 42 and 54 (partially) and claims 5 to 7, 43 and 44 (completely).

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**Supplemental Box**

(To be used when the space in any of Boxes I to VIII is not sufficient)

**Continuation of Box No: II, Observations where unity of invention is lacking.**

The international application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept. In coming to this conclusion the International Searching Authority has found that there are different inventions as follows:

1. Claims 1 to 4, 35 to 42 and 54 (partially) and claims 5 to 7, 43 and 44 (complete) are directed towards plants of the Gramineae family (grasses) transformed to modulate production of polyhydroxyalkanoate. It is considered that transformation of grasses for modulation of production of polyhydroxyalkanoate comprises a first "special technical feature".
2. Claims 1 to 4, 35 to 42 and 54 (partially) and claims 8, 9 and 45 (complete) are directed towards plants of the Gramineae family (grasses) transformed to modulate production of p-hydroxybenzoic acid (pHBA). It is considered that transformation of grasses for modulation of production of pHBA comprises a second "special technical feature".
3. Claims 1 to 4, 35 to 42 and 54 (partially) and claims 10, 11 and 46 (complete) are directed towards plants of the Gramineae family (grasses) transformed to modulate production of vanillin. It is considered that transformation of grasses for modulation of production of vanillin comprises a third "special technical feature".
4. Claims 1 to 4, 35 to 42 and 54 (partially) and claims 12 to 15 and 47 (complete) are directed towards plants of the Gramineae family (grasses) transformed to modulate production of sorbitol. It is considered that transformation of grasses for modulation of production of sorbitol comprises a fourth "special technical feature".
5. Claims 1 to 4, 35 to 42 and 54 (partially) and claims 16, 17 and 48 (complete) are directed towards plants of the Gramineae family (grasses) transformed to modulate production of indigo. It is considered that transformation of grasses for modulation of production of indigo comprises a fifth "special technical feature".
6. Claims 1 to 4, 35 to 42 and 54 (partially) and claims 18 to 20 and 49 (complete) are directed towards plants of the Gramineae family (grasses) transformed to modulate production of fructan. It is considered that transformation of grasses for modulation of production of fructan comprises a sixth "special technical feature".
7. Claims 1 to 4, 35 to 42 and 54 (partially) and claims 21, 22 and 50 (complete) are directed towards plants of the Gramineae family (grasses) transformed to modulate production of lactic acid. It is considered that transformation of grasses for modulation of production of lactic acid comprises a seventh "special technical feature".
8. Claims 1 to 4, 35 to 42 and 54 (partially) and claims 23 to 28 and 51 (complete) are directed towards plants of the Gramineae family (grasses) transformed to modulate production of adipic acid. It is considered that transformation of grasses for modulation of production of adipic acid comprises an eighth "special technical feature".

**Supplemental Box**

(To be used when the space in any of Boxes I to VIII is not sufficient)

**Continuation of Box No: II, Observations where unity of invention is lacking.**

9. Claims 1 to 4, 35 to 42 and 54 (partially) and claims 29 to 32 and 52 (complete) are directed towards plants of the Gramineae family (grasses) transformed to modulate production of 1,3-propanediol. It is considered that transformation of grasses for modulation of production of 1,3-propanediol comprises a ninth "special technical feature".
10. Claims 1 to 4, 35 to 42 and 54 (partially) and claims 33, 34 and 53 (complete) are directed towards plants of the Gramineae family (grasses) transformed to modulate production of 2-phenylethanol. It is considered that transformation of grasses for modulation of production of 2-phenylethanol comprises a tenth "special technical feature".

These groups are not so linked as to form a single general inventive concept, that is, they do not have any common inventive features, which define a contribution over the prior art. The common concept linking together these groups of claims is the transformation of plants from the Graminaea family for modulating levels of production of a metabolic or biosynthetic product. However this concept is not novel in the light of a large number of documents of which the following is a selection:

WO 01/95691 (Southern Cross University *et al*) 20 December 2001  
WO 01/95702 (Southern Cross University *et al*) 20 December 2001  
WO 01/49852 (Vicente-Carbajosa *et al*) 12 July 2001  
US 20020042930 (Botha *et al*) 11 April 2002  
US 20010053847 (Tang *et al*) 20 December 2001  
US 2002061570 (Dekalb Genetics Corp) 23 May 2003  
US 5 792 921 (Londesborough *et al*) 11 August 1998  
WO 98/49332 (Pioneer Hi-Bred International) 5 November 1998

Therefore these claims lack unity a posteriori.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU03/00903

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in<br>Search Report |              | Patent Family Member |               |
|---|--------------|----------------------|---------------|
| US 6 091 002                              | AU 31443/97  | US 5 958 754         | US 2003028917 |
|   | CA 2 259 251 | US 5 959 179         | WO 98/00557   |
|   | US 5 942 660 | US 6 228 623         |               |
| WO 00/52183                               | AU 35161/00  | US 6 448 473         |               |
|   | EP 1 159 435 | US 2003182678        |               |
| WO 01/23596                               | AU 77412/00  |                      |               |
| END OF ANNEX                              |              |                      |               |